AD_____

AWARD NUMBER DAMD17-98-1-8468

TITLE: Combined Use of Tissue Morphology, Neural Network Analysis of Chromatin Texture & Clinical Variables to Predict Prostate Cancer Agressiveness from Biopsy Mater

PRINCIPAL INVESTIGATOR: Alan W. Partin, M.D., Ph.D.

CONTRACTING ORGANIZATION: The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205-2196

REPORT DATE: October 1999

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 1999	Annual Summary (01 Oct 98 - 30 Sep 99)

4. TITLE AND SUBTITLE
Combined Use of Tissue Morphology, Neural Network Analysis of Chromatin Texture & Clinical Variables to Predict Prostate Cancer Agressiveness From Biopsy Material

5. FUNDING NUMBERS
DAMD17-98-1-8468

6. AUTHOR(S)
Alan Partin, M.D., Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The Johns Hopkins University School of Medicine
Baltimore, Maryland 21205-2196

e-mail: Apartin@jhmi.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
This report contains colored photos

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release;
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 Words)*

PURPOSE: To combine clinical, serum, pathological and computer derived information into an artificial neural network to develop/validate a model to predict prostate cancer tumor aggressiveness in both a retrospective and prospective cohort of men with clinically localized prostate cancer both prior to and after radical prostatectomy.

SCOPE: Prospective enrollment of 500 men who are scheduled to undergo radical prostatectomy (year 01). Development of a artificial neural network model (year 02). Prospective validation of this model (projected year 03). All models will be tested and developed for biopsy and prostatectomy material.

MAJOR FINDINGS: To date, we have completed prospective enrollment of 527 men, collected tissue, serum and clinical/pathological information for 387 and completed computer image data analysis of 171 samples. No model has been developed to date and awaits final enrollment. We anticipate final prospective data to be complete and model developed by 1/4/2000. At this time we will begin enrollment of prospective validation samples.

14. SUBJECT TERMS	15. NUMBER OF PAGES
Prostate	25
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

_____ Where copyrighted material is quoted, permission has been obtained to use such material.

_____ Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

_____ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

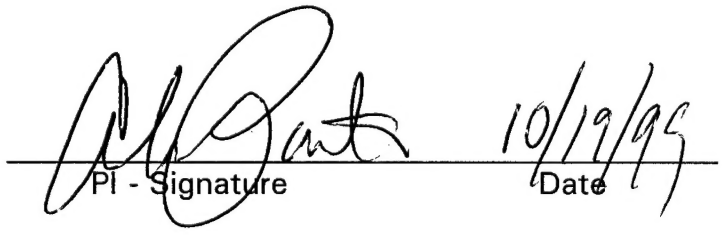
_____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, national Research Council (NIH Publication No. 86-23, Revised 1985).

X For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

_____ In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

_____ In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

_____ In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.



PI - Signature 10/19/95 Date

Table of Contents

Introduction:

Several specific objectives were outlined for our research proposal entitled *Combined use of Tissue Morphology, Neural Network Analysis of Chromatin Texture and Clinical variables to Predict Prostate Cancer Aggressiveness from biopsy Material.* We proposed to combine standard prognostic methods (clinical stage, PSA, Gleason score, and biopsy information) with Neural Network analysis of chromatin texture and computer derived tissue morphology prospectively to predict pathologic stage. We also intended to retrospectively investigate in prostatectomy specimens using a similar combination of clinical, histologic and computer derived characteristics to predict disease recurrence following surgery. This resulting technology and nuclear analysis would then be applied to study a group of men with long term follow-up after surgery to develop and validate this technology in predicting recurrence following surgery. Lastly, we intended to use this methodology to develop and validate an accurate model for predicting time to metastatic progression/death after biochemical recurrence. With these specific objectives outlined, a statement of work was submitted detailing the task and time line necessary to accomplish the goals of the proposal. Task one of our statement of work outlined the steps involved in the prospective enrollment of 500 men for prediction of pathologic stage model development. Completion of this objective was projected for 9 months following the initiation of this project. Below are the initial steps outlined in Task one, followed by an update of our progress to date.

Body: Specific aims

A. Identification and prospective enrollment of consecutive radical prostatectomy cases performed at the Johns Hopkins Hospital.

527 patients have been enrolled with 476 successfully fulfilling all inclusion criteria.

B. Obtain tissue blocks for each case.

Tissue blocks have been obtained for 387 of the 476 patients admitted into this research study.

C. Cut and prepare histologic sections.

Histologic sections have been obtained from 387 cases.

D. Measure nuclear features with the QNG model.

Image analysis has been completed on 171 cases.

E. Enter all clinical, pathological, and quantitative nuclear data into the computer.

Clinical and pathological data for 527 patients has been collected and organized into a relational database.

F. Multivariate analysis to determine optimal prognosis prediction model.

Analysis of the first 527 patients began this month, October 1999.

Task two of our approved statement of work details the steps necessary for prospective enrollment of 400 men for pathologic stage model validation. This portion of the project has a projected completion of 13 months following project initiation.

To date, only 476 men have passed all inclusion criteria and been admitted to the study. Enrollment will continue until approximately 500 men are admitted. Multivariate analysis of this subset is not slated to begin until this portion of task one is complete. Therefor, the specific initiatives of task two will begin following this analysis and pathologic stage model construction. We anticipate completion of this model by month 16 (January 2000) and thus will begin prospective enrollment of the 400 men model validation subset by month 18 (March 2000).

Task three of the research proposal outlines the steps involved in predicting tumor aggressiveness from biopsy/prostatectomy specimens. This portion of the statement of work should be completed by month 14 of the study. Our progress to date is indicated below:

A. Obtain tissue blocks from 300 cases treated at Johns Hopkins with radical prostatectomy.
300 pathological specimens have been identified. The blocks are currently being collected and should be complete by month 15. (December 1999)

B. Cut histologic sections and prepare slides for QNG analysis.
This portion of task three will be conducted following completion of section A with an anticipated date of completion of month 19 (April 2000).

C. QNG determinations
Refer to task 3, section B comment.

D. Tissue morphology analysis.
Refer to task 3, section B comment.

F. Enter clinical data, pathological information, QNG results and tissue morphology into a database.
Clinical and pathological data for 300 patients has been collected and organized into a relational database.

G. Calculate model for prediction of post-operative progression from prostatectomy specimens.
This step will be completed following collection of all data involved with task three. Anticipated completion of this initiative is month 19 (April 2000).

Task four involves validation analyses from prostatectomy specimens for prediction of tumor aggressiveness. Our initial statement of work projected completion of this portion of the

project by month 30 (March 2001). The identification and analysis of these additional 200 prostatectomy specimens will begin immediately following the tumor aggressiveness model construction detailed in task three. We believe that completion of this initiative will be prior to month 30 deadline initially proposed.

Lastly, task five of this research study involves retrospective development of a model for prediction of development of metastasises/death following biochemical recurrence following surgery. This task involves identification of 300 men who have exhibited biochemical or metastatic recurrence following surgery. We anticipate beginning this final portion of the project in month 16 (January 2000).

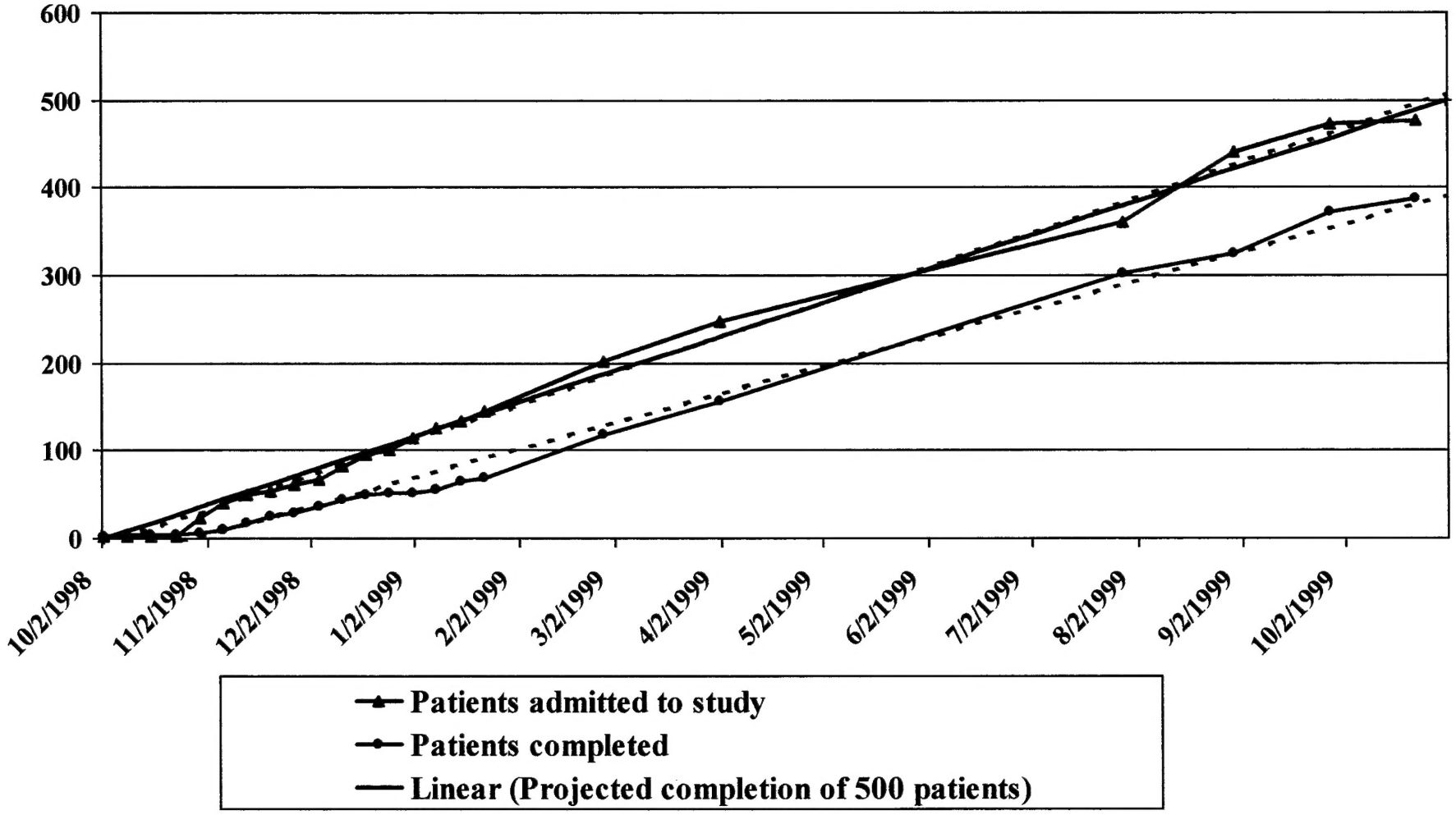
Research accomplishments:

- Prospective enrollment of 527 patients. (See attached graph)
- Biopsy material obtained on 418 patients.
- Histology completed on 278 cases.
- Image analysis completed on 171 cases.

Reportable outcomes:

- Manuscript in press. Reference: Steven R. Potter, M. Craig Miller, Leslie A. Mangold, Kerrie A. Jones, Jonathan I. Epstein, Robert W. Veltri, and Alan W. Partin. *Genetically Engineered Neural Networks for Predicting Prostate Cancer Progression after Radical Prostatectomy*, Submitted June 1999. (Attached)

Progress Report: DOD
Date: 10-22-99



The blue line charts a course for completion of 500 patients (enrollment through slides sent to UroCor for analysis) on Oct 31, 1999. The dotted lines depict current trend lines for enrollment and completion of patients. Patients admitted to study 476 (51 patients were additionally admitted but excluded), of those 387 have been completed.

Appendix B

Rapid Communication

GENETICALLY ENGINEERED NEURAL NETWORKS FOR PREDICTING PROSTATE CANCER PROGRESSION AFTER RADICAL PROSTATECTOMY

June 14, 1999

STEVEN R. POTTER [1,†], M. CRAIG MILLER [2], LESLIE A. MANGOLD [1], KERRIE A. JONES [1], JONATHAN I. EPSTEIN [1,3], ROBERT W. VELTRI [2], AND ALAN W. PARTIN [1]

[1] The Brady Urological Institute, The Johns Hopkins Hospital, Baltimore, MD; [2] UroCor, Inc., UroSciences Group, Oklahoma City, OK; [3]Department of Pathology, The Johns Hopkins Hospital, Baltimore, MD.

†, Resident, The Brady Urological Institute, The Johns Hopkins Hospital

Word Count: Abstract-327; Text-1660

Grant Support: SPORE Grant CA58236; KOCH Foundation Grant; Department of Defense Grant # 98-05-15-02

Running Title: NEURAL NETWORKS TO PREDICT PROSTATE CANCER PROGRESSION

Key Words: prostate cancer, progression markers, quantitative nuclear grade (QNG), neural networks, computational algorithms

Address Proofs/Reprint Requests: *Alan W. Partin M.D, Ph.D. The Johns Hopkins Hospital, Brady Urological Institute Department of Urology 600 North Wolfe St. Baltimore MD 21287*

ABSTRACT

Objectives. To use pathologic, morphometric, DNA ploidy, and clinical data to develop and test a genetically engineered neural network (GENN) for the prediction of biochemical (prostate specific antigen (PSA)) progression after radical prostatectomy for a select group of men with clinically localized prostate cancer.

Methods. Two-hundred and fourteen men who underwent anatomic radical retropubic prostatectomy (RRP) for clinically localized prostate cancer were selected based on adequate follow-up, pathologic criteria indicating an intermediate risk of progression, and availability of archival tissue. The median age was 58.9 years (range 40-87 years). Men with Gleason score 5-7 and clinical stage T1b-T2c tumors were included. Follow-up was a median of 9.5 years. Three GENNs were developed using pathologic findings (Gleason score, extra-prostatic extension, surgical margin status), age, quantitative nuclear grade (QNG), and DNA ploidy. These networks were developed using three randomly selected training (n=136) and testing sets (n=35). Different variable subsets were compared for ability to maximize prediction of progression. Both standard logistic regression and Cox regression were used concurrently to calculate progression risk.

Results. Biochemical (PSA) progression occurred in eighty-four (40%) men, with a median time to progression of 48 months (range 1-168 months). GENN models were trained using inputs consisting of: 1) pathologic features and patient age; 2) QNG and DNA ploidy; and 3) all variables combined. These GENN models achieved average accuracy's of 74.4%, 63.1%, and 73.5% respectively for prediction of progression in the training sets. In the testing sets, the three GENN models had accuracy's of 74.3%, 80.0%, and 78.1% respectively.

Conclusions. The GENN models developed show promise in predicting progression in select groups of men after radical prostatectomy. Neural networks using QNG and DNA ploidy as inputs performed as well as networks using Gleason score and staging information. All GENN models were superior to logistic regression modeling and to Cox regression in prediction of PSA progression. Development of models using improved input variables and imaging systems in larger, well-characterized patient groups with long-term follow-up is ongoing.

Introduction

Improvements in prostate cancer staging have dramatically increased the percentage of men presenting with clinically localized disease [1,2]. However, 30-40% of men undergoing RRP will suffer biochemical (PSA) progression within 10 years [1]. Estimates of progression risk are based on tumor volume, surgical margin status, Gleason score and pathologic stage. [2,3,4,5]. Nuclear morphometry and DNA ploidy provide additional variables for use in predictive models [4,5,6,7]. Improvements in our ability to predict progression after definitive therapy are needed to help patients and physicians decide whether and when to initiate adjuvant therapy.

Statistical tools, such as logistic regression, have routinely been used to analyze data and predict treatment outcomes [2-6,8]. However, the variability and complexity of the data may exceed the capacity of standard modeling methods. Artificial neural networks (ANNs) attempt to simulate human decision-making using adaptation and inference parameters [9]. Neural networks can better define non-linear patterns between predictor variables and previously unknown outcomes than linear statistical models.

Validation of a neural network requires separate training and testing phases. In the training phase, the ANN "learns" the relationships of input and outcome and assigns weights to the input variables. Once these weights are formalized, the ANN is considered "trained." The ANN must then be validated on a different data set. The term "genetic" in GENN refers to a method of network development in which network architecture is determined by the data presented to it [10]. The GENN develops the relationships between input variables and outcome, selects for the "fittest" solutions, and ultimately "evolves" an optimal network. Use of ANNs in urologic oncology has shown promise [11-13].

Previously, we used logistic regression to evaluate the ability of QNG and Gleason score to predict progression after RRP [5]. We determined that QNG and Gleason score stratified patients into low-, moderate-, and high-risk groups for prostate cancer progression. In follow-up to that retrospective study, we now compare the ability of GENNs and logistic regression in predicting progression in a subset of RRP patients in which accurate prediction is especially difficult.

MATERIAL AND METHODS

PATIENTS

A total of 214 men with prostatectomy Gleason score 5-7 and clinical stage T1b-T2c cancers were non-consecutively selected from a cohort of over 1800 RRP patients treated between 1982 and 1996 at one institution [14]. Selection of these men was based upon: 1) adequate follow-up (≥5 years in non-progressors), 2) complete clinical data and, 3) availability of archival tissue. All men underwent anatomic radical retropubic prostatectomy. Men with seminal vesicle invasion or lymph node involvement discovered at surgery were excluded because of known high risk of progression. Men who underwent adjuvant or neoadjuvant hormonal or radiation therapy were also excluded, as the natural history of prostate cancer in these men could not be ascertained. Most were treated before the availability of pre-operative PSA testing. These 214 men formed the training and testing groups for development and analysis of three GENN models, and had a minimum follow-up among non-progressors of 5 years (range 5-16 years). All preoperative clinical, pathologic, and postoperative data were gathered prospectively, and are summarized in Table 1.

Men were followed with serum PSA measurements at 3-month intervals for one year, at 6-month intervals for an additional year and yearly thereafter (after PSA became available in 1987). Annual interview and DRE were performed. Biochemical recurrence was defined as a postoperative serum PSA >0.2 ng/ml. No patient received radiation or hormonal therapy before biochemical disease recurrence.

ACQUISITION OF IMAGE DATA

Representative sequential 5 um-thick sections were cut from archival, formalin-fixed, paraffin-embedded tissue. Alternating sections were stained with hematoxylin and eosin (H&E) and Feulgen reagents, and areas of cancer marked [15]. Approximately 150 nuclei from each tumor were analyzed. Forty-one nuclear morphometric descriptors (NMD's) were measured for each image, including 11 DNA content, 22 Markovian texture, and 8 nuclear shape features [5].

NEURAL NETWORK ANALYSIS

All data were analyzed using NeuroGenetic Optimizer™ (NGO) v2.6 software (BioComp Systems, Inc., Redmond, WA), that builds predictive models using genetic algorithms. Input variables included prostatectomy pathology (Gleason score, extra-prostatic extension, surgical margin status), age, DNA ploidy, and QNG (the variance of 41 different NMDs). These variables were classified as nominal (extra-prostatic extension, margin status), categorical (Gleason score, DNA ploidy), or continuous (age and NMDs).

Using pathology and age (model #1), QNG and DNA ploidy (model #2), or a combination of all variables (model #3), we constructed three randomly selected training and testing sets balanced for the number of progressors (n=84) and non-progressors (n=87) in our cohort. The training sets consisted of 80% of the balanced sample while the testing sets utilized the remaining 20% of the balanced sample. The same three training and testing sets were employed for network analysis and logistic regression. To avoid network overfitting, each network was limited to a maximum of 200 training iterations.

STATISTICAL ANALYSIS

All data were analyzed with *Stata*™ v5.0 statistical analysis software (Stata Corporation, College Station, TX). Logistic regression (LR) was used to evaluate the accuracy of the various GENNs. The outcome variable was biochemical progression. Receiver operator characteristic (ROC) curves and the areas under the curves (AUC) were calculated for each of the GENN models, as were sensitivity, specificity, and accuracy. Accuracy was defined as the overall percentage of cases that were correctly classified. Kaplan-Meier analysis was performed using the average results of model #3. Actuarial curve significance was determined using the log-rank test of equality and Wilcoxon-Gehan test.

LR was performed concurrently on the same three randomly selected training and testing sets using the same combinations of input variables. A multivariate significance stringency of $p < 0.25$ was used for backwards stepwise LR. Again, ROC curves and AUC's were calculated for each model, and sensitivity, specificity, and accuracy calculated. The Cox proportional hazards model was performed on the training and testing set output of model #3.

RESULTS

Among the 149 (70%) tumors with extra-prostatic spread at pathologic staging, 66 (31%) also had positive margins. The remaining 65 (30%) tumors were organ-confined. Over a median follow-up of 9.5 years, eighty-four (40%) men developed biochemical progression within a median of 4 years (range 1 - 14 years). In the biochemical progression-free men (n=130), 75% of the tumors had prostatectomy Gleason scores of 5 or 6, while of men with biochemical progression (n=84), 67% had a prostatectomy Gleason score of 7.

The three GENN models achieved average accuracy's of 74.4%, 63.1%, and 73.5% for predicting progression in the training sets. The testing sets produced average accuracy's of

74.3%, 80.0%, and 78.1%, respectively (Table 2). The use of QNG and DNA ploidy alone as input variables (model #2) had a lower sensitivity and higher specificity than use of pathology results and patient age (model #1). The training and testing sets were analyzed concurrently by logistic regression and Cox proportional hazards modeling (Table 3). Logistic regression maximized performance in the training sets while the GENN models maximized performance in the testing sets. For the testing set, Cox analysis yielded a sensitivity of only 39%, specificity of 67%, and accuracy of 53 % (Table 3).

Kaplan-Meier analysis, performed on the average outputs of model #3 for the entire patient sample, allowed stratification of tumors into four biochemical recurrence likelihood risk groups (Figure 1). The log-rank test of equality was used to calculate significance levels for the differences between risk groups (p-value between groups I-II, 0.092; II-III, <0.0001; III-IV, 0.0113).

DISCUSSION

Although PSA testing has revolutionized the early detection of prostate cancer, PSA levels alone have a limited ability to predict progression. Prediction is especially problematic in men with clinically organ-confined cancer who, at surgery, have Gleason score 5-7 tumors and negative seminal vesicles and lymph nodes [14].

We developed and tested neural networks and compared them to the results of logistic regression in a selected cohort of men at intermediate risk of cancer progression and with lengthy follow-up. Our findings suggest that GENNs are useful in progression prediction, and may aid in clinical decision making and the rational design of clinical trials. All GENN testing-set models were superior to logistic regression in predicting progression. Progression prediction

using a Cox regression model was inferior to neural network performance. Development of three different GENN models allowed comparison of different input variables.

The use of neural networks in predicting outcome after surgery shows promise, but some limitations are apparent. Currently, a pathologist and imaging technician are required to select cancer nuclei for QNG determination. The utility of QNG (models #2, #3) was reduced by limitations of the nuclear imaging system used. Analysis with current state-of-the-art systems is ongoing and will likely improve the contribution of QNG in these models.

Because of limitations on patient numbers necessitated by our desire for lengthy follow-up and intermediate progression risk, we did not construct a separate set of previously unstudied patients to serve as a validation cohort. This does not invalidate comparison of GENN and LR results. Because the testing set patients were not used to adjust the input weights in our networks, the testing set results are useful in assessing these networks as tools for predicting progression. The collection of a validation patient cohort is underway.

The absence of PSA values as input variables, necessary because the length of follow-up achieved meant that most men had surgery before the PSA era, was potentially limiting. However, new input variables, such as PSA or other serological, immunohistochemical, or molecular markers, can be incorporated into GENNs with relative ease, and are likely to increase their predictive value. Few of these men had stage T1c lesions, and development of predictive models using a more representative percentage of nonpalpable cancers is ongoing.

CONCLUSIONS

The application of neural networks to progression prediction shows promise in men at intermediate risk of progression in whom prediction has historically been most inaccurate. GENN creation is a logical step in the development of progression modeling. Networks were developed with high sensitivity and specificity for prediction of prostate cancer progression in a group of men with long-term prospective follow-up after RRP. Improvements in nuclear imaging systems and input variable selection promise further improvements. Development of these improved models in larger, well-characterized patient groups with long-term follow-up is ongoing. Further development of GENNs will provide improved prognostication after radical prostatectomy, allowing early and appropriate evaluation of investigational adjuvant therapies.

REFERENCES

1. Merrill RM, and Brawley OW: Prostate cancer incidence and mortality rates among white and black men. *Epidemiology* 8:126-131, 1997
2. Kattan MW, Eastham JA, Stapleton AMF, Wheeler TM, Scardino PT: A preoperative nomogram for disease recurrence following radical prostatectomy for prostate cancer. *J Natl Cancer Inst 90(10):766-771, 1998*
3. Partin AW, Piantadosi S, Sanda MG, Epstein JI, Marshall FF, Mohler JL, Brendler CB, Walsh PC, Simons JW: Selection of men at high risk for disease recurrence for experimental adjuvant therapy following radical prostatectomy. *Urology* 45:831-38, 1995
4. Badalament RA, Miller MC, Peller PA, Young DC, Bahn DK, Kochie P, O'Dowd GJ, and Veltri RW: An algorithm for predicting non-organ confined prostate cancer using the results obtained from sextant core biopsies and prostate specific antigen level. *J Urol* 156:1375-1380, 1996
5. D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Schnall M, Tamaszewski JE, Wein A: A multivariate analysis of clinical and pathological factors that predict for prostate specific antigen failure after radical prostatectomy for prostate cancer. *J Urol* 154:131-138, 1995
6. Veltri RW, Miller MC, Partin AW, Coffey DS, and Epstein JI: Ability to predict biochemical progression using Gleason score and computer-generated quantitative nuclear grade derived from cancer cell nuclei. *Urology* 48:685-691, 1996
7. Veltri RW, O'Dowd GJ, Orozco R, and Miller MC: The role of biopsy pathology, quantitative nuclear morphometry, and biomarkers in the pre-op prediction of prostate cancer staging and prognosis. *Semin Urol Onc* 16(3):106-117, 1998

8. Partin AW, Kattan MW, Subong ANP, et al: Combination of prostate-specific antigen, clinical stage, and Gleason score to predict pathological stage of localized prostate cancer: A multi-institutional update. *JAMA* 277:1445-1451, 1997

9. Kattan MW, Cowan ME, and Miles BJ: Computer modeling in urology. *Urology* 47:14-21, 1996

10. Forrest S: Genetic algorithms: Principles of natural selection applied to computation. *Science* 261:872-878, 1993

11. Snow PB, Smith DS, and Catalona WJ: Artificial neural networks in the diagnosis and prognosis of prostate cancer: a pilot study. *J Urol* 152(5):1923-1926, 1994

12. Stotzka R, Manner R, Bartels PH, and Thompson D: A hybrid neural and statistical classifier system for histopathologic grading of prostatic lesions. *Anal Quant Cytol Histol* 17:204-218, 1995

13. Tewari A and Narayan P: Novel staging tool for localized prostate cancer: a pilot study using genetic adaptive neural networks. *J Urol* 160(2):430-436, 1998

14. Pound CR, Partin AW, Epstein JI, Walsh PC: Prostate-specific antigen after anatomic radical retropubic prostatectomy: patterns of recurrence and cancer control. *Urol Clin North Am* 24:395-406, 1997

15. Bacus SS, Bacus JW, Bacus JV, Chin DM, Ortiz R, Stern RK, Morrow DW: Prostate adenocarcinoma: An image analysis technique for DNA ploidy determination. *Lab Med* 24:225-231, 1994

List of Tables and Figures (4 Total); Figure Caption

Table 1. Summary of demographic and clinical data in 214 men presenting with clinically localized prostate cancer.

Table 2. Results of GENN models on randomly selected training (n=136) and testing (n=35) sets balanced for the number of progressors vs. non-progressors.

Table 3. Results of logistic regression and Cox proportional hazard regression models on randomly selected training (n=136) and testing (n=35) sets balanced for the number of progressors vs. non-progressors.

Figure 1. Kaplan-Meier analysis of the average of the outputs for the entire patient sample (n=214) using the trained model #3 GENN. The patients are separated into four distinct biochemical (PSA) progression likelihood risk groups. Group I, GENN<0.30 (n=23) p=0.0925; Group II, 0.30≤GENN<0.50 (n=78) p<0.0001; Group III, 0.50≤GENN<0.70 (n=92); Group IV, ≥0.70 (n=21). The p-values between groups I and II = 0.092; Groups II and III <0.0001; Groups III and IV = 0.0113).

Table 1. Summary of demographic and clinical data in 214 men presenting with clinically localized prostate cancer.

Average Age:	58.9 ± 6.4 (range = 40 - 87) yrs
Average Follow-Up Time:	7.8 ± 3.9 (range = 1 - 16) yrs
Average Time To Progression:	4.5 ± 3.3 (range = 1 - 14) yrs
Average Follow-Up (Non-Prog):	9.9 ± 2.7 (range = 5 - 16) yrs

Clinical Stage	N (%)
T1b - T1c	6 (3%)
T2a	72 (33%)
T2b	113 (53%)
T2c	23 (11%)

Prostatectomy Gleason Scores	N (%)
5	50 (23%)
6	75 (35%)
7	89 (42%)

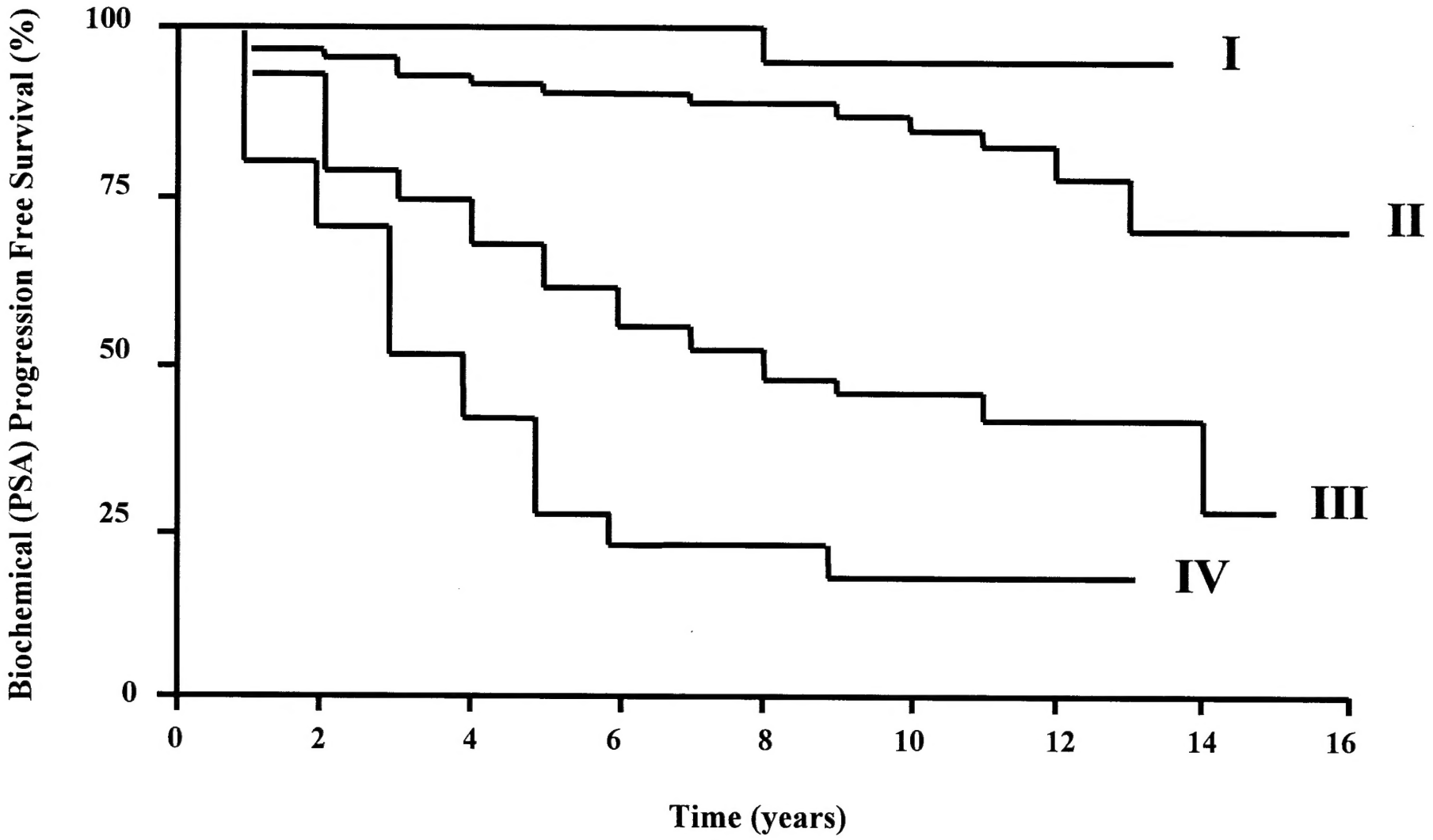

Biochemical (PSA) Progression Free Survival (%)
100
75
50
25
0
0
2
4
6
8
10
12
14
16
Time (years)
I
II
III
IV

Table 2. Results of GENN models on randomly selected training (n=136) and testing (n=35) sets balanced for the number of progressors vs. non-progressors.

		MODEL#1	MODEL #2	MODEL #3
		Pathology + Age*	NMD's + DNA Ploidy*	All Variables Combined*
Average for Random Training Sets (n=136)	Sensitivity	83.6 ± 0.0%	53.7 ± 6.5%	75.1 ± 2.3%
	Specificity	65.5 ± 2.5%	72.4 ± 7.4%	71.8 ± 3.9%
	Accuracy	74.4 ± 1.2%	63.1 ± 6.3%	73.5 ± 0.8%
	AUC	79.4 ± 2.1%	68.3 ± 5.8%	79.6 ± 0.9%
Average for Random Testing Sets (n=35)	Sensitivity	88.2 ± 5.9%	74.5 ± 9.0%	84.3 ± 9.0%
	Specificity	61.1 ± 11.1%	85.2 ± 3.2%	72.2 ± 0.0%
	Accuracy	74.3 ± 4.9%	80.0 ± 2.9%	78.1 ± 4.4%
	AUC	71.3 ± 8.6%	74.0 ± 4.0%	73.5 ± 7.5%

* Average ± Standard Deviation

Table 3. Results of backwards stepwise regression models on randomly selected training (n=136) and testing (n=35) sets balanced for the number of progressors and nonprogressors. (Cutoff >= 0.50 for each Model)

		Logistic Regression			Cox Regression
		Model 1: Pathology + Age*	*Model 2*: QNG + DNA Ploidy*	*Model 3*: All Variables Combined*	*Model 3*: All Variables Combined*
Average for Random Training Sets (n=136)	Sensitivity	83.6 ± 0.0%	74.1 ± 3.1%	85.6 ± 2.3%	72.1 ± 6.0%
	Specificity	65.5 ± 2.5%	74.3 ± 4.8%	86.4 ± 3.5%	88.3 ± 3.0%
	Accuracy	74.4 ± 1.2%	74.2 ± 3.9%	86.0 ± 2.7%	80.3 ± 4.4%
	AUC	79.8 ± 1.7%	83.0 ± 1.4%	93.7 ± 1.2%	89.2 ± 1.1%
Average for Random Testing Sets (n=35)	Sensitivity	68.6 ± 3.4%	56.9 ± 12.2%	56.9 ± 9.0%	39.2 ± 41.3%
	Specificity	64.8 ± 6.4%	68.5 ± 6.4%	59.3 ± 3.2%	66.7 ± 36.4%
	Accuracy	66.7 ± 4.4%	62.9 ± 7.6%	58.1 ± 4.4%	53.3 ± 1.7%
	AUC	68.0 ± 5.8%	64.7 ± 7.3%	64.7 ± 3.0%	54.9 ± 9.9%

* Average ± Standard Deviation